

REMARKS

This Amendment and Response is filed in response to the Office Action dated June 18, 2002. Applicant's attorney appreciates the careful attention given the present application and provides the above referenced amendments and following remarks in order to place the case in condition for final allowance. While Applicant's attorney respectfully disagrees with the Examiner's position with regard to the restriction requirement, it is noted that this requirement was deemed proper by the Examiner and made final. Therefore, Claims 1-25 and 35-58 have been deleted from the present application as they are no longer under consideration. These claims are being removed solely to facilitate allowance of the claims currently being considered, and claims of a similar or identical scope may be pursued in this or later filed application. In this Amendment and Response, claims 26, 29, 30, and 34 are amended, claims 27, 28, 31, 32, and 33 remain pending, and new claims 59-67 have been added. Accordingly, claims 26-34 & 59-67 are under consideration.

Initially, with regard to the objection to the specification for informalities, e.g. failing to append sequence ID's, applicants attorney has attempted amend each occurrence of a cited sequence of a nucleotide or polypeptide with a "SEQ ID NO:". Accordingly, it is respectfully submitted that the objections to the specification should be withdrawn. It should also be noted by the Examiner that the claims have been broken down into "methods of inhibiting" and "methods of preventing" to avoid any confusion as a result of the use of the alternative language in original claim 26

Turning to the substantive portion of the Office Action, the Examiner has rejected claims 29, 30 and 34 under 35 USC §112 for purportedly failing to particularly point out and distinctively claim the subject matter which applicant regards as the invention. Each of the issues raised by the Examiner with regard to claims 29, 30 and 34 have been addressed by amendment and it is respectfully submitted that the rejection of claims 29, 30 and 34 for failing to particularly point out distinctively claim the subject matter which applicant regards as his invention should be withdrawn.

Claims 26-34 have been rejected for failing to satisfy the written description requirement of 35 USC§112. It is respectfully submitted that in light of the amendments to the claims, that this rejection is no longer applicable and should be withdrawn. The claims, as

amended, are drawn to inhibiting or preventing a viral infection by introducing a Phospholipid Scramblase polypeptide, or any fragment thereof, containing an amino acid sequence PPxY. The viral infection is not as broadly drawn as indicated by the Examiner. Rather, the viral infection is the type that is at least partially enveloped by a membrane envelope derived from the host cell. Furthermore, while the Examiner states that the instant disclosure does not teach specific fragments of Phospholipid Scramblase that contain this motif, Applicant has cited numerous examples of Phospholipid Scramblases which contain this motif and has provided strong scientific support that one ordinarily skilled in the art would expect fragments containing PPxY to function similarly. The ubiquitous PPxY sequence in viral species and Scramblase species which inhibit viral budding provides adequate support for such a claim. Certainly, one ordinarily skilled in the art would be able to synthesize and/or isolate polypeptides having such activity. Applicant is not required, nor is he encouraged, to describe each and every structure which may contain this motif but rather provide a number of species within a genus to support such a claim. Applicant has done this by providing numerous examples. Accordingly, the rejection of claims 26-34 under 35 USC§112 for failing to satisfy written description should be withdrawn.

Claims 26-34 have also been rejected under 35 USC§112 for failing to enable one skilled in the art to which it pertains to make or use the invention. It is respectfully submitted that, in light of the current amendments, and a closer reading of the specification, that this rejection should be withdrawn. As amended, the claims are not drawn to a method of inhibiting or preventing any infection of a virus, but rather those viral infections that are virus-budding viral infections (i.e., enveloped by a membrane envelope derived from the host cell). This is a discreet class of viruses and the claims are limited to the recited viruses. For the Examiner's convenience, enclosed with this Response is a portion of Fields "Virology" Volume 1, pg. 24, which describes a variety of virus families and characterizes them as being enveloped or non-enveloped. In addition, the current claims are directed to Phospholipid Scramblase that prevent or inhibit viruses that function by being enveloped by a membrane envelope derived from the cell. The Phospholipid Scramblase appear to have a conserved sequence PPxY. While it is true the claims are intended to cover yet undiscovered viruses which function via the PPxY binding motif or the L binding motif, this is entirely proper and is irrelevant with regard to enablement. Applicant has provided a road-map, and given specific examples, of Phospholipid Scramblase sequences or fragment thereof having a PPxY binding motif suitable for use in the present

invention. One ordinarily skilled in the art has available numerous animal models to determine whether unknown Phospholipid Scramblases or fragments thereof have a PPxY sequence and whether they would function as inhibitors of viral infection.

The Examiner also states that there is not a teaching in the specification that addresses the concern for effectively delivering the Phospholipid Scramblase without interrupting normal cellular function. Delivery means are described in the Specification and are well known in the art. Specifically, applicant has introduced Phospholipid Scramblase by means of transferring a DNA encoding the protein into cells.

The Examiner also states that there is not a teaching in the specification that addresses the concern for recognizing how any fragment containing the PPxY could be recognized as inhibiting or preventing viral infections. Contrary to this, the specification teaches how Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), antiviral function can be determined. One of ordinary skill in the art could use similar tests without undue experimentation to determine the antiviral function of other Phospholipid Scramblase fragments or peptidomimetics with other viruses. Such tests could be performed on the PLSCR fragment or peptidomimetics and virus as taught in the specification with and without a cytokines like interferon.

The Examiner states that it was not clear from Example 4 whether inhibition of VSV was due to the Scramblase or addition of interferon. Contrary to this however, Example 4 teaches that at a multiplicity of infection of 10, Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), expressing cells (IFN- β with Hu PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2)) had a viability of 33% compared to cell viability of only 7% in vector control cells (IFN- β without Hu PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2)). The example shows inhibition of viral activity due to the Phospholipid Scramblase. The virus yield study of Example 4 also demonstrate the cooperative effect of IFN- β , and Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), for reducing viral yield. Without IFN- β , Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), results in a smaller suppression of viral yield; together IFN- β and Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), results in a 10 fold reduction in yield. The cooperative effect observed in this example may or may not be observed with other cytokines and other Phospholipid Scramblases. However the teachings of this example would enable those skilled in the art to ascertain the antiviral function of Phospholipid Scramblase, its fragments, or peptidemimetics thereof with and without cytokines.

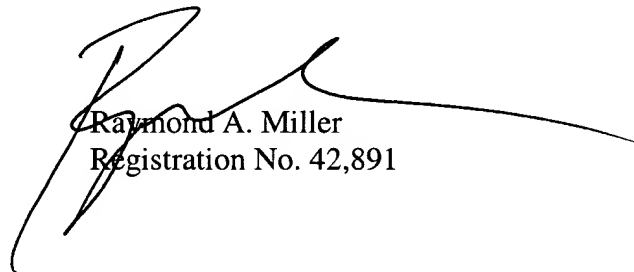
For all the reasons advanced above, Applicant respectfully submits that the rejections have been overcome and should be withdrawn. Applicant respectfully submits that the application is in condition for allowance. Prompt favorable action is respectfully and earnestly solicited.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for this Amendment, or credit any overpayment, to deposit account no. 50-0436.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKING TO SHOW CHANGES MADE

IN THE SPECIFICATION:

[0037] (ONCE AMENDED) Figure 11 shows gene structure of the HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) gene. Top: Schematic showing location of intron-extron borders within the HuPLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), genomic sequence of approx. 28 kb. Exons are represented by vertical lines. Below: Location of exons within the cDNA are indicated by arrows. Genomic sequence for HuPLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), deposited under GenBank accession no. AF224492.

[0046] (ONCE AMENDED) Despite over forty years of research on IFNs, there are substantial gaps in our understanding of the molecular mechanisms that are responsible for the biological effects of IFNs. For example, triply deficient mice lacking RNase L, PKR and Mx1 can still mount a very substantial residual anti-viral effect in response to IFN- α . We have recently identified phospholipid scramblase-1 (PLSCR1)- a plasma membrane protein- as a new member of the IFN-regulated gene family. The purpose of the proposed studies is to gain an understanding of the cellular and plasma membrane changes induced through IFN's transcriptional upregulation of PLSCR1, and how these changes mediated through PLSCR1 potentially relate to the biologic activity of IFN in vivo. [As will be detailed in SECTION C, we have recently identified phospholipid scramblase-1 (PLSCR1)--a plasma membrane protein--as a new member of the IFN-regulated gene family. The purpose of the proposed studies is to gain an understanding of the cellular and plasma membrane changes induced through IFN's transcriptional upregulation of PLSCR1, and how these changes mediated through PLSCR1 potentially relate to the biologic activity of IFN in vivo.]

[0050] (ONCE AMENDED) During the past three years, we have made considerable progress in defining potential molecular mechanisms underlying this Ca^{2+} -induced transbilayer movement of plasma membrane PL [(research conducted under R01 HL36946)]. We reported the purification and characterization of an integral erythrocyte membrane protein ([[]]designated "PL scramblase; hu PLSCR1"(SEQ ID NO: 1 and SEQ ID NO: 2))[] that, when reconstituted in liposomes, mediates a Ca^{2+} -dependent and pH-dependent accelerated transbilayer movement of all PL, mimicking the reorganization of plasma membrane PL observed either upon elevation of [][Ca^{2+}]_c [] or upon acidification of the cytosol. There is also evidence that the same protein mediates similar function in platelets. The properties of this protein in PL bilayer membranes indicate that PLSCR1 is responsible for accelerated transbilayer movement of PS and other plasma membrane PL in all cells and tissues exposed to elevated [][Ca^{2+}]_c, arising as a consequence of immune injury or agonist-induced cell activation, and potentially, during cell senescence (*see below*).

Paragraph [0051] (ONCE AMENDED) The deduced sequence of human PLSCR1 reveals a proline-rich acidic protein (35.1 kD; pKa=4.85) with a single predicted transmembrane domain near the C-terminus. There is also a single potential protein kinase C phosphorylation site (Thr¹⁶¹) and an apparent EF-hand related Ca^{2+} -binding motif (see below). Analysis of the cDNA-derived protein sequence predicts a strongly preferred inside-to-outside orientation of the predicted 19-residue transmembrane domain, consistent with a type 2 plasma membrane protein. Thus, the bulk of the protein is predicted to extend from the cytoplasmic membrane leaflet, leaving a short extracellular tail. The predicted orientation of this protein is consistent with the anticipated topology of PL scramblase in erythrocytes and platelets, where the lipid-mobilizing function is responsive to Ca^{2+} or to acidification (pH<6.5) only at the endofacial surface of the plasma membrane. Northern blotting revealed that PLSCR1 mRNA was present in a variety of hematological and non-hematological cells and tissues. We have identified the residues in PLSCR1 that function in binding Ca^{2+} and our data suggest that the activity of PLSCR1 is also regulated post-translationally through palmitoylation at one or more Cys thiols. We have recently

identified three additional members of the PL scramblase gene family (hu PLSCR 2-4 (SEQ ID NOS: 3, 4, 5, 6, 7 and 8)) and the putative PL scramblase orthologues in mouse (mu PLSCR1-4 (SEQ ID NOS: 9, 10, 11, 12, 13, 14, 15 and 16)) have been identified.

Paragraph [0052] (ONCE AMENDED) We have shown that PLSCR1 is induced in IFN treated cells where it localizes to the plasma membrane, the site of budding for these viruses. The N-terminal regions of hu and mu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2 and SEQ ID NO: 9 and SEQ ID NO: 10) share with diverse types of membrane bound viruses late function PPxY motifs required for release of virus particles from cells. PPxY motifs in PLSCR1 suppress virus budding by competing with the viral M or Gag proteins for binding to cellular WW domain proteins. This is the first example of host mimicry of a viral protein for the purpose of suppressing virus infections.

Paragraph [0055] (ONCE AMENDED) A potential link between PLSCR1 gene expression and neoplastic cell transformation was recently suggested by Kasukabe and associates. They describe a gene transcript (designated **NOR1**) that is markedly down-regulated in transformed murine monocytic cell lines relative to its expression in normal blood monocytes, and, a 5'-truncated form of this same transcript (designated **TRA1**) expressed only in leukemogenic mouse monocytic cell lines (but not expressed in normal monocyte or non-leukemogenic monocytic cell lines). Butyrate induction of monocytes to macrophages was accompanied by induced expression of the NOR1 transcript^[28]. They suggest that the truncated TRA1 gene product is associated with leukemogenesis in vivo, whereas increased NOR1 expression is associated with macrophage differentiation. Analysis of the open reading frame predicted by the NOR1 and TRA1 cDNA sequences reveals near-identity of protein sequence with hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), in overlapping portions of each polypeptide. This suggests that NOR1 (expressed in normal mouse monocytes and other tissues) is the murine orthologue of human PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) (mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10)) whereas the TRA1 gene product, found only in leukemogenic cell lines, is a truncated form of mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) that arises through alternative splicing (deleting exons 1-5). Down-regulation of wild-type mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) (i.e. NOR1) in transformed monocytes and

the de novo expression of the alternatively-spliced, truncated form of this protein (i.e., TRA1) in only leukemogenic subclones, suggests that NOR1 (and thus presumably PLSCR1) is required for normal cell senescence, whereas mutant TRA1 might promote leukemogenic potential, potentially as a dominant-negative PL scramblase inhibitor.

Paragraph [0056] (ONCE AMENDED) As was noted, we now have shown that the expression of hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) is markedly upregulated by IFN. PLSCR1 directly contributes to the antiproliferative action of IFN and provides a causal explanation for the observed association of aberrant NOR1/TRA1 gene expression in transformed and leukemogenic cell lines.

Paragraph [0135] (ONCE AMENDED) Analysis of 5' flanking genomic sequence in reporter constructs showed that transcriptional control of PLSCR1 was entirely regulated by a single IFN-stimulated response element (ISRE) located in the first exon. A similar induction of PLSCR1 by IFN- α 2a was also observed in a variety of other human tumor cell lines as well as in human umbilical vein endothelial cells. In these cell lines, the marked increase in PLSCR1 expression by IFN- α 2a was not accompanied by increased cell surface exposure of PS. These results suggest that remodeling of the cell surface requires both exposure to IFN and a second yet-to-be identified event to stimulate plasma membrane PL scramblase activity and to mobilize PS to the cell surface. We have recently confirmed that the IFN-inducibility observed for hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) is also shared by the murine orthologue, mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10). Experiments to identify the ISRE(s) responsible for IFN-regulated expression of mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) gene (as described for hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2)) are now in progress.

Paragraph [0136] (ONCE AMENDED) To directly measure the effect of PLSCR1 induction on tumor growth in the absence of other IFN induced proteins, we constitutively expressed hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA in the human ovarian cancer cell line, HEY1B. The hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA was subcloned under the control of a CMV promoter in plasmid vector, pcDNAneo3. Transfection of the HEY1B cells was followed by selection in media containing G418. Western blots probed with a

monoclonal antibody to PLSCR1 (mab 4D2) revealed that only one of fifty clones expressed high levels of PLSCR1. The clone, S48, expressed about 4-fold more PLSCR1 than the parental cells. This compares with the 10-fold increase in PLSCR1 levels obtained with IFN- α treatment of the cells. The in vitro growth rates of the S48 cells and of the clonal cell line (V24) containing the empty vector was determined in the presence and absence of 1,000 units per ml of IFN- α 2a. While the growth of both cell lines was modestly suppressed by IFN, there was no difference in the growth rates of the V24 and S48 cells. In sharp contrast, there was a dramatic difference in the ability of the two cell lines to form tumors after being implanted into nude mice. In these experiments, the V24 cells and the PLSCR1 expressing clone, S48, (10^6 cells/site) were injected subcutaneously (s.c.) into the flanks of groups of six nude mice. Tumor growth was monitored every 3-4 days with a caliper and the excised tumors were measured upon termination of the experiment. The tumor growth rate of the empty-vector control clone was about 8-fold higher than that of the PLSCR1 (S48) clone. To rule out clonal variation as the cause of the differences, we cloned hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA into vector pIREShyg (Clontech) which expresses a bicistronic mRNA under the control of a CMV promoter. The first open reading frame is PLSCR1 followed by an internal ribosome entry site (IRES) and a hygromycin B phosphotransferase sequence. Therefore, after transfection of the HEY1B cells and selection in hygromycin-containing media, expression of PLSCR1 was tightly coupled to hygromycin resistance. The result was high expression (about 4- to 10-fold over basal levels) of PLSCR1 in every clone analyzed and as well as in the pool of selected cells (data not shown). The pools of PLSCR1 expressing cells and the empty-vector pool of cells were inoculated s.c. into the flanks of groups of nude mice. The tumor results obtained from the uncloned pooled cells (transfected with PLSCR1 cDNA) were similar to those obtained for the S48 clone, thus eliminating a clonal artefact as the basis for the anti-tumor activity of PLSCR1 (data not shown). In this proposal we will investigate the molecular and cellular mechanism of the anti-tumor effect of PLSCR1.

Paragraph [0137] (ONCE AMENDED) To determine the possible antiviral function of PLSCR1, cell viability and viral yield assays with VSV were performed in the presence or absence of IFN- β on cells expressing hu PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), cDNA. VSV is a rhabdovirus containing a negative RNA genome with five genes in the order 3'-

N-P-M-G-L-5'. Its virions are composed of two main parts, a nucleocapsid or ribonucleoprotein (RNP) core and a lipid bilayer envelope. VSV was chosen for these studies because IFN inhibits its replication at a late stage and it is a membrane-bound virus with a PPxY viral budding motif in its M protein. Hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) is an IFN-induced membrane protein that contains two PPxY motifs near its cytoplasmic N-terminus. Therefore, PLSCR1 could interfere with VSV budding or assembly by competing with VSV M protein for binding to cellular WW domain proteins required for these late viral processes.

Paragraph [0140] (ONCE AMENDED) BLAST analysis of the GenBank EST database using hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA revealed three different families of EST clones that were similar, but distinctly different from the sequence we originally reported for hu PL scramblase (PLSCR1), (SEQ ID NO: 1 and SEQ ID NO: 2). In order to obtain cDNAs for these putative homologues of huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), the relevant EST clones were used to design PCR primers. Full length cDNAs were obtained by PCR using cDNA from multiple human tissues as templates. As illustrated, the cloned cDNAs encode three novel proteins with high homology to huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) (FIG. 9). The predicted open reading frames of these putative homologues show sequence identities to huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) of 59% (huPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4); 224 AA; GenBank AF159441), 47% (huPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6); 295 AA; GenBank AF159442) and 46% (huPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8); 329 AA; GenBank AF199023), respectively. Corresponding cDNAs of putative murine orthologues of each of the four hu PLSCR (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8) family members have also been identified, and similar proteins of unknown function are also predicted in the C. elegans, Drosophila, and porcine genome (data not shown).

Paragraph [0141] (ONCE AMENDED) Inspection of the four human PLSCR (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8) homologues reveals a low degree of similarity for the proline-rich N-terminal portion of the proteins (AA 1 to 85 in huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2)), and highest degree of identity towards the C-terminus. This includes a highly conserved segment (AA 273 to 284 in huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2)) which has been shown in huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) to contain the Ca²⁺-binding site. The

sequence of huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) predicts a type II membrane protein with single transmembrane domain near the C-terminus (AA 291-309), and most of the polypeptide (AA 1-290) extending into the cytosol. By contrast, such predictions are ambiguous for the newly described homologues, as are predictions of putative intracellular localization. Of note, the predicted open reading frame for huPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4), the closest homologue to huPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), is missing the proline-rich N-terminus that is characteristic for all other members of this family. As was discussed, this segment in PLSCR1 that is missing in PLSCR2 contains PPxY and PxxP motifs which may serve as binding sites for proteins containing WW or SH3 domains, respectively, and potentially confer on PLSCR1 anti-viral activity. The PPxY and PxxP motifs common to hu PLSCR1, PLSCR3, & PLSCR4 (SEQ ID NOS: 1, 2 5, 6, 7 and 8) are also conserved in the corresponding mouse orthologue (FIG. 10) as well as in the potential porcine (GENBank F14810) and *C. elegans* (GENBank Z82084, AF078785) orthologues of PLSCR1. Although binding partners for any of the PLSCR proteins have not been identified to date, it is interesting to note that the functional implication of the missing N-terminal segment in huPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) may also be a potential loss of interaction with an adaptor or signaling molecule. A similar truncation deleting the proline-rich segment of mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) has previously been associated with leukemogenesis.

Paragraph [0142] (ONCE AMENDED) *Chromosomal assignment of PL scramblase family members.* Chromosomal localization by analysis of STS sequences of the NCBI Human Gene Map'99 and/or radiation panel hybrid mapping revealed that the genes for hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), hu PLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4), and PLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) are tightly clustered between markers D3S1557 and D3S1306 (164.6-168.3 cM) on chromosome 3 (3q23) at the physical location 537.09 cR₃₀₀₀ (P130). HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) was also mapped to chromosome 3 at 3q23 by fluorescence in situ hybridization. By contrast, PLSCR3 maps to chromosome 17 (p13.1) between markers D17S1828 and D17S786 (9.8-18.1 cM) at the physical location 53.50 cR₃₀₀₀ (P0.90).

Paragraph [0143] (ONCE AMENDED) *Tissue Distribution of PL Scramblase Family Members.* Initial insight into the tissue distribution of the four newly-identified members of the PLSCR gene family was obtained by Northern blotting with ³²P-labeled probes specific for huPLSCR1 to huPLSCR4 (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8), respectively. Transcripts for PLSCR1 (~2400 bp and ~1600 bp) were expressed in spleen, thymus, prostate, testis, uterus, small intestine, colon, peripheral blood lymphocytes, heart, placenta, lung, liver, kidney and pancreas, but below the limits of detection in brain and skeletal muscle. By contrast, PLSCR2 (~1600 bp) was detected only in testis. PLSCR3 (~2400 bp and ~1600 bp) was detected in spleen, thymus, prostate, uterus, small intestine, colon, PBL, skeletal muscle, heart, placenta, lung, kidney and pancreas, but not in testis, brain or liver. PLSCR4 (~3600 bp) was detected in all tissues examined except peripheral blood lymphocytes, and was the only PLSCR family member detected in brain.

Paragraph [0144] (ONCE AMENDED) *Antibody Probes of PLSCR Family Members.* We have several monoclonal antibodies specific for hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) that do not cross-react with hu PLSCR2-4 (SEQ ID NOS: 3, 4, 5, 6, 7 and 8). Two of the mabs have been found to cross-react with mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) and can be used to selectively monitor expression of the protein in murine cells and tissue. In order to develop additional antibody probes, peptides corresponding to unique sequence identified in the various PLSCR homologues have been synthesized and peptide-KLH conjugates injected into rabbits for antisera production.

Paragraph [0145] (ONCE AMENDED) These antisera were analyzed by ELISA and Western blotting against recombinant PLSCR1-4 produced in E. coli as MBP-fusion proteins. At the present time, we have available high titer antisera selective for hu and mu PLSCR1-3 (SEQ ID NOS: 1, 2, 3, 4, 5 and 6) and (SEQ ID NOS: 9, 10, 11, 12, 13 and 14). Immunizations with peptides derived from hu and mu PLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) and (SEQ ID NO: 15 and SEQ ID NO: 16) are now in progress. Thus, we anticipate that antibody reagents suitable for monitoring selective protein expression of each of the four PLSCR family members in both human and mouse cells and tissues will be available [prior to start of YEAR 1 of the proposed Project].

Paragraph [0146] (ONCE AMENDED) Under contract between Scripps/BCSEW and Lexicon Genetics, Inc., we initiated genomic cloning and Cre-Lox targeted disruption of the murine PLSCR gene locus in 1998. This was prior to our discovery that PLSCR is a multigene family of proteins in both mouse and man, currently shown to include four expressed genes. We now recognize that the original mouse orthologue of human PL scramblase that we had cloned and targeted for gene disruption is mu PLSCR2 (SEQ ID NO: 11 and SEQ ID NO: 12), not the true orthologue of hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2). We therefore recently cloned and targeted disruption of the mu PLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) gene (see FIG. 10). In both cases (PLSCR1 & PLSCR2), the targeting construct was designed to disrupt exon 8, which (by sequence alignment to hu PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2)) is predicted to contain the Ca²⁺ binding and putative transmembrane segments of the proteins. The PLSCR2 knockout was completed last year and the breeding colonies transferred to Scripps for use in this Project. To date, no abnormality has been identified in the homozygote PLSCR2.^{-/-} animals. As of date of submission of this application (01/ 29/00) matings of the chimeric mice containing the PLSCR1 gene disruption were performed, and 14 resulting agouti pups are now being analyzed for germline transmission. [We anticipate that breeding colonies will be transferred to Scripps Transgenic facility in the Spring of '00 for use in this Project.] No information is now available on the viability or phenotype of the homozygous PLSCR1^{-/-} animals.

Paragraph [0147] (ONCE AMENDED) Cloning of Human PL Scramblase 1 Gene. A BAC-human genomic library (Genome System Inc., St. Louis) was screened with a 1.445 kb HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA probe (GenBank accession number AF098642) by hybridization. A positive clone of approximately 100 kb was obtained, digested with EcoRI, and the fragments were cloned into pcDNA3 (Invitrogen). Subclones were identified by hybridization with digoxigenin-labeled HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA probe, and DNA inserts were sequenced on an ABI DNA Sequencer Model 373 Stretch (Applied Biosystems) using PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer).

Paragraph [0148] (AMENDED) Construction of 5' Flanking Region Deletions of PLSCR1 Gene. A 4180 bp DNA fragment consisting of the 5'flanking region (0 to -4120) and the first 60 bp of the first exon (+1 to +60) of the HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) gene was cloned into pGL3-basic-luciferase reporter vector (Promega). In order to identify the promoter region of the gene, the 5' flanking DNA was serially deleted both from the 5' and the 3' end by PCR-mediated deletion and cloned into pGL3-basic-luciferase reporter vector for analysis.

Paragraph [0149] (AMENDED) Cell Culture and Transfection of Daudi Cells. The Burkitt's B cell lymphoma cell line Daudi was cultured in RPMI 1640 complete medium with 20% fetal bovine serum, 100 U of penicillin/ml, and 100 µg of streptomycin/ml, at 37 °C., 5% CO₂. Cells were washed and suspended to 1.35×10^7 /ml in OPTI-MEM. To 0.8 ml of cell suspension in a 0.4 cm electroporation cuvette, 20 µg of pGL3-5'flanking region or deletions of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) and 20 µg of pSV-β-galactosidase (Promega) were added. The mixture was incubated for 10 min on ice, and electroporated at 380 V and 500 µF using a Bio-Rad Gene Pulser II (Bio-Rad). After incubation for 10 min at 37. °C. the cells were plated in 10 ml of RPMI complete medium. Forty-eight hours later, transfected cells were harvested for luciferase and β-galactosidase assay.

Paragraph [0151] (ONCE AMENDED) Cloning of PL Scramblase Family Members. Blast search of the GenBank database of expressed sequence tags (EST) with HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) cDNA identified three distinct clusters of EST clones each displaying overlapping identities. Appropriate EST clones were obtained from American Type Culture Collection, sequenced, and the information was used to design PCR primers specific for the 3' and 5' ends of the various homologues. Full length cDNAs were obtained by PCR using a human erythroleukemia cell (HEL) cDNA library (Clontech; for HuPLSCR2 and HuPLSCR3 (SEQ ID NOS: 3, 4, 5 and 6)) or human multiple tissue cDNA (Clontech; pancreas; for HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8)) as template. Each PCR reaction and cloning was performed in triplicates, and Advantage HP₂ DNA polymerase mix (Clontech) was used to decrease PCR-mediated error. PCR products were cloned into pCR2.1 (Invitrogen) for sequencing.

Paragraph [0152] (ONCE AMENDED) Chromosomal Mapping. The chromosomal location for HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) was determined using the GeneBridge 3 Human Radiation hybrid panel and oligonucleotides 5'-CCTGGTGCTTAGGGTAGACAATATG-3' and 5'-CTGACGTCCTGGGTAGAAGGCCTGGG-3' as the forward and reverse primers, respectively (Research Genetics, Huntsville, AL). The primers flank a small intron (88 bp) within the 5' untranslated region of HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4), giving a PCR product of 314 base pairs. The map position was calculated using the Stanford server [<http://www-shgc.stanford.edu>] (on the world wide web at URL address:-shgc.Stanford.edu).

Paragraph [0153] (Once Amended) Tissue Distribution. Human multi-tissue Northern blots (Clontech) were hybridized to random prime labeled cDNA probes of each HuPLSCR (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8) family member. The HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) probe consisted of the 5' 498 bp of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) (gbAF098642). The HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) probe (1265 bp) was prepared by digesting EST clone AA813518 with Not1 and Xho1, and the HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) probe (851 bp) by digesting EST clone N78598 with Not1 and Xho1. The cDNA fragments were separated from vector sequences by agarose gel electrophoresis and purified using Wizard columns (Promega). The cDNA probes were labeled with α -³²P-dATP (50 μ Ci/25 ng cDNA, 3000 Ci/mmol; ICN) using the random prime labeling kit from Boehringer Mannheim to a specific activity $\geq 1 \times 10^9$ dpm/ μ g. Due to non-specific hybridization of the cDNA probe, an RNA antisense probe was designed for HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6). A PCR product of the 3' untranslated region of HuPLSCR3, (SEQ ID NO: 5 and SEQ ID NO: 6), was prepared using the forward primer 5'-TGTGAGGAGACCATCACCTCGAC-3' and reverse primer 5'-AAAGCTGATATGCCTGTGTGCC-3'. The reverse primer contained the T7 promoter sequence (5'-AATTTAATACGACTCACTATAGGG-3') at the 5' end. The PCR product was purified using the Qiaquick PCR purification kit (Qiagen). A α -³²P-labeled antisense RNA probe was prepared using 50 ng of the PCR product as template in T7 transcription reaction with α -³²P-UTP (800 Ci/mmol; 20 μ Ci/ μ l; Amersham) following the instructions included in the T7 Strip-EZ RNA kit (Ambion). Multi-tissue Northern blots were

prehybridized for 1 hour at 68 °C. in ExpressHyb hybridization buffer (Clontech) followed by hybridization for 18 hours at 68 °C. in the same buffer containing 2×10^6 cpm/ml denatured random prime-labeled probe. For HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6), the blots were prehybridized in Ultrahyb hybridization buffer (Ambion) with 100 µg/ml denatured salmon sperm DNA and 50 µg/ml yeast RNA and hybridized in the same buffer containing ^{32}P -labeled antisense RNA probe (2×10^6 cpm/ml) at 68 °C. for 18 hours. The blots were washed at a final stringency of 0.1.times. SSC in 0.1% SDS at 50 °C. (68 °C. for HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6)), and exposed to Amersham Hyperfilm MP.

Paragraph [0154] (Once Amended) Human PLSCR1 Gene Structure. In order to gain insight into the gene organization of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), a clone of approximately 100 kb of genomic DNA was obtained from a BAC-human genomic library by screening with a HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) Cdna probe. EcoR1 digested fragments were cloned into pcDNA3, and sequence from six different clones was used to deduce approximately 30 kb of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) genomic DNA. The organization of the gene was deduced by alignment of the genomic sequence with Cdna sequence for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) (GenBank accession number AF098642). The HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) gene consists of 9 exons, 8 introns and 5' flanking sequence (deposited under GenBank accession numbers AF153715 and AF224492). As shown in Table 1, invariant gt and ag were found at the intron splice donor and acceptor sites. As illustrated in FIG. 1, the first exon is untranslated, with the open reading frame starting in exon 2. Of interest, Kasukabe et al. [(16)] reported the occurrence of a truncated form of MuPLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) (termed MmTRA1a), the closest murine orthologue of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), in a mouse monocytic cell line which was highly leukemogenic when injected into syngeneic or athymic mice. In addition, non-leukemogenic sublines became leukemogenic when transfected with MmTRA1a. By contrast, normal macrophages expressed only full length MuPLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10). Comparison of the sequence of MmTRA1a with FIG. 1 reveals that murine MmTRA1a is likely a product of alternative splicing, as the predicted open reading frame reported by Kasukabe et al. [(16)] starts at a position corresponding to the beginning of Exon 6 in HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2). It remains to be determined whether the analogous

alternatively spliced forms of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) are similarly associated with leukemias in man.

Paragraph [0155] (ONCE AMENDED) Promoter Analysis. In order to identify the promoter region for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), luciferase reporter constructs of 5' flanking sequence and serial 5' or 3' deletions were expressed in Daudi cells. As illustrated by the data in FIGS. 2 and 3, a reporter construct containing 5' untranslated sequence comprised of -4120 to +60 exhibited strong promoter activity. Deletion of sequence from the 5' end from -4120 bp to -557 bp did not affect promoter activity (FIG. 2). However, deletion from -95 bp of 5' flanking sequence to +60 bp of the first (untranslated) exon resulted in the loss of more than 97% of promoter activity, locating the promoter of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) to that region (FIG. 3). Computer analysis of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) 5' flanking sequence using the MatInspector V2.2 program [<http://www.gsf.de/biodv/matinspector.html>] (on the world wide web at URL address: [gsf.de/biodv/matinspector.html](http://www.gsf.de/biodv/matinspector.html)) revealed two GC boxes (TAGGGGAGGGGCCT at -79 bp to -66 bp, and AGGAGGTGGGCGCA at -59 bp to -46 bp) and a CCAAT box (TCTCTCCAATG at -111 bp to -101 bp) (FIG. 4), consistent with the data in FIG. 3 locating promoter activity to that region. In addition, potential binding sites for transcriptional activators, including activator protein 4 (AP4, upstream stimulating factor (USF), eukaryotic transcriptional regulator 1 (ETS1), interferon-stimulated response element (ISRE), and interferon regulatory factor (IRF), were identified. We had previously identified the single ISRE that is located in the first untranslated exon (+21 to +35) as the primary site responsible for the upregulation of HuPLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), by interferon- α .

Paragraph [0156] (ONCE AMENDED) Identity of a Novel PL Scramblase Gene Family. Upon performing BLAST searches of the GenBank EST database with human PL scramblase 1 (HuPLSCR1) (SEQ ID NO: 1 and SEQ ID NO: 2), we noted three distinct clusters of EST clones that were similar, but distinctly different from the sequence we had originally reported for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2). In order to obtain cDNAs for these putative homologues of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), sequence derived from relevant EST clones was used to design PCR primers. Full length cDNAs were obtained by PCR using a cDNA library from human erythroleukemia cells (HEL), and cDNA from multiple

human tissues as template. As illustrated in FIG. 5, the cloned cDNAs encode three novel proteins with high homology to HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2). The predicted open reading frames encode proteins with 59% (HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4); 224 AA; GenBank AF159441), 47% (HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6); 295 AA; GenBank AF159442) and 46% (HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8); 329 AA; GenBank AF199023) identity, respectively, to HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2). Furthermore, cDNAs of novel murine orthologues of HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6) (MuPLSCR3 (SEQ ID NO: 13 and SEQ ID NO: 14); 327 AA; GenBank AF159850) and HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) (MuPLSCR4 (SEQ ID NO: 15 and SEQ ID NO: 16); partial, in progress) have been cloned and sequenced (data not shown). Closer inspection of the four human PLSCR (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8) homologues reveals low degree of similarity for the proline-rich aminoterminal portion of the proteins (amino acids 1 to 85 in HuPLSCR1) (SEQ ID NO: 1 and SEQ ID NO: 2), and highest degree of identity towards the carboxyterminus, including a region (AA 273 to 284 in HuPLSCR1, SEQ ID NO: 1 and SEQ ID NO: 2) which has been shown for huPLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), to contain a Ca^{2+} binding site required for the Ca^{2+} -induced transmembrane movement of phospholipids. We have previously noted that computer analysis of HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) predicts a type II protein with a transmembrane domain near the carboxyterminus (AA 291-309), and most of the polypeptide (AA 1-290) extending into the cytosol. By contrast, such predictions are ambiguous for the newly described homologues, as are predictions of putative intracellular localization. Of note, the predicted open reading frame for HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4), the closest homologue to HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2), is missing the proline-rich aminoterminal that is characteristic for all other members of this family. As pointed out previously, this region also contains a number of PXXP motifs which may serve as potential binding sites for proteins containing SH3 domains. In addition, HuPLSCR1, 3, and 4 (SEQ ID NOS: 1, 2, 5, 6, 7 and 8) all contain one or more PPxY motifs, suggesting a potential interaction with proteins containing WW domains. Such domains are primarily found in proteins with signaling or regulatory function. Although binding partners for any of the PLSCR proteins have not been identified to date, it is interesting to note that the functional implication of the missing aminoterminal segment in HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) may be a potential loss of interaction with an adaptor or signaling molecule. A

similar truncation has previously been noted to confer leukemogenic potential to MuPLSCR1 (SEQ ID NO: 9 and SEQ ID NO: 10) (MmTRA1a).

Paragraph [0157] (ONCE AMENDED) Chromosomal Assignment of PL Scramblase Family Members. The chromosomal locations of HuPLSCR1, HuPLSCR3 and HuPLSCR4 (SEQ ID NOS: 1, 2, 5, 6, 7 and 8), were determined from nucleotide sequence homologies to STS sequences found on the NCBI Human Gene Map'99 [<http://www.ncbi.nlm.nih.gov/genemap/>] (on the world wide at the URL address: [ncbi.nlm.nih.gov/genemap/](http://www.ncbi.nlm.nih.gov/genemap/)). The genes for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) (stSG10277) and HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) (gb N78598/G37067) are clustered between markers D3S1557 and D3S1306 (164.6-168.3 Cm) on chromosome 3 (3q23) at the physical position 537.09 Cr₃₀₀₀. HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) has also been independently mapped to chromosome 3 at 3q23 by fluorescence in situ hybridization. A partial sequence for the HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6) gene is located between nucleotide 10501 and 9174 of the gene sequence deposited in GenBank under gb AF097738, which also codes for a non-receptor tyrosine kinase gene (nucleotides 531-9180). The non-receptor tyrosine kinase gene has been localized to chromosome 17p13.1 between markers D17S1828 and D17S786 (9.8-18.1 Cm) at the physical position 53.50 Cr₃₀₀₀, thus localizing HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6) to that position. The gene for HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) was mapped as described in *Experimental Procedures*, and was found to be located on chromosome 3 (3q23), closely clustered with HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) and HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8), suggesting that these three homologues arose by gene duplication.

Paragraph [0158] (ONCE AMENDED) Tissue Distribution of PL Scramblase Family Members. The tissue distribution for the four members of the PL scramblase family of proteins was evaluated by Northern blotting with ³²P-labeled probes specific for HuPLSCR1 to HuPLSCR4 (SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8), respectively. The specificity of the probes was ascertained by DNA dot blot (FIG. 6). Whereas amounts of mRNA for HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) in many of these tissues were below the limit of detection, the mRNA for the other three homologues were expressed in most of the 16 different tissues

examined. However, the expression patterns for these three family members show distinct differences. FIG. 7 shows that mRNA for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) was below the limits of detection in brain and skeletal muscle. As previously reported, two different size transcripts (~2.55 kb and 1.6 kb) were detected for HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) in all tissues expressing this gene. Kasukabe et al. have suggested that the different size transcripts arise from alternative polyadenylation signals within the 3' untranslated region of the HuPLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) gene. Interestingly, the expression of HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) mRNA appears to be highly restricted. Although trace amounts of HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) could be amplified from HEL cells through several rounds of PCR for sequencing purposes (see Experimental Procedures), a 1.6 kb message was only detected in testis. This result was confirmed by probing a human Multi Tissue Expression Array (Clontech, Cat. #7775-1), which again yielded a positive blot against mRNA of testis only. This blot also revealed that in addition to the tissues listed in FIG. 7, HuPLSCR2 (SEQ ID NO: 3 and SEQ ID NO: 4) message was also not detected in any tissues of the gastrointestinal tract, bladder, ovary, lymph node, bone marrow, and adrenal, thyroid, salivary or mammary gland (results not shown). HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6) mRNA was below the limit of detection in testis, brain or liver. Two sizes of mRNA were detected with the HuPLSCR3 (SEQ ID NO: 5 and SEQ ID NO: 6) specific probe: whereas a 1.8 kb mRNA species was observed for most tissues, a .about.2.1 kb mRNA transcript was detected in skeletal muscle. An mRNA transcript of 4 kb was detected for HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) in all tissues examined except peripheral blood lymphocytes. Importantly, HuPLSCR4 (SEQ ID NO: 7 and SEQ ID NO: 8) mRNA was the only family member expressed at detectable levels in brain tissue. Whether HuPLSCR1, 3, and 4 (SEQ ID NOS: 1, 2, 5, 6, 7 and 8) have redundant function in a number of tissues, or whether these proteins exhibit activities that are distinct for each family member is the subject of future experimentation.

Page 56, Line 1 (ONCE AMENDED)

Table 1 Hu-PLSCR1, (SEQ ID NO: 1 and SEQ ID NO: 2), exon and intron sequences.

Paragraph [0165] (ONCE AMENDED) Molecular Cloning of 5' Flanking Region of PLSCR1 Gene and Construction of Deletions. Human PLSCR1 (SEQ ID NO: 1 and SEQ ID NO: 2) gene was cloned from a BAC-human genomic library (Genome System Inc., St. Louis, Mo.) using full length PLSCR1 cDNA for hybridization, and 4.12 kb of 5'flanking region was sequenced (GenBank AF153715). 4.18 kb DNA consisting of the 5'flanking region (-1 to -4120) and the first 60 bp of the first exon of the gene (+1 to +60) was amplified by PCR using Advantage DNA polymerase mix (CLONTECH Laboratories, Inc., Palo Alto, Calif.), and PCR products were cloned into pGL3-basic-luciferase reporter vector (Promega, Madison, Wis.). Analysis of the 5' flanking region for the presence of putative binding sites for transcription factors was performed using MatInspector V2.2. The four putative binding sites for ISGF3 or IRFs (see FIG. 5) were deleted by PCR-mediated deletion. All DNA sequencing was performed on an ABI DNA Sequencer Model 373 Stretch (Applied Biosystems) using PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, Calif.).

In the Claims:

Claims 1 through 25 are deleted.

The following claims are amended as indicated:

26. (ONCE AMENDED) A method of inhibiting [or preventing] viral infection comprising, introducing [into viral-infected cells or uninfected cells] into cells a Phospholipid Scramblase polypeptide or fragment[s] thereof contain[ing]s the amino acid sequence PPxY and prevents virus budding.

29. (ONCE AMENDED) The method of claim 26, wherein the [virus] viral infection functions by being enveloped by a membrane envelope derived from the cell[is an infection of a membrane bound virus].

30. (ONCE AMENDED) The method of claim 26, wherein the [polypeptides] Phospholipid Scramblase polypeptides and fragments thereof bind to proteins containing one or more WW domain sequence motifs.

34. (ONCE AMENDED) The method of claim 26, wherein the fragments are peptidomimetics of the Phospholipid Scramblase polypeptides.

Claims 35 through 58 are deleted.

Please add new claims 59-67:

59. (NEW) A method of preventing viral infection comprising, introducing a Phospholipid Scramblase polypeptide or fragments thereof into a cell, wherein said Phospholipid Scramblase polypeptide or fragments thereof contains the amino acid sequence PPxY and prevents virus budding.

60. (NEW) The method of claim 59, wherein the viral infection is an infection of a virus selected from the group consisting of a rhabdovirus, a filovirus, a retrovirus, a flavivirus, a coronavirus, a orthomyxovirus, a hubyavirus, a hepadnavirus, a herpesvirus, a poxvirus, a togavirus, a iridovirus, a paramyxovirus and a arenavirus.

61. (NEW) The method of claim 60, wherein the viral infection is selected from the group consisting of an HIV infection, an Ebola virus infection, a Marburg virus infection, and a Rabies virus infection.

62. (NEW) The method of claim 59, wherein the viral infection functions by being enveloped by a membrane envelope derived from the cell.

63. (NEW) The method of claim 59, wherein the Phospholipid Scramblase polypeptides and fragments bind to proteins containing one or more WW domain sequence motifs.

64. (NEW) The method of claim 59, wherein the Phospholipid Scramblase polypeptide is interferon-inducible.

65. (NEW) The method of claim 64, wherein the Phospholipid Scramblase polypeptide has the amino acid sequence as set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

66. (NEW) The method of claim 59, further comprising administering an interferon.

67. (NEW) The method of claim 59, wherein the fragments are peptidomimetics of the Phospholipid Scramblase polypeptides.